

SUPPLEMENTAL TABLES

SUPPLEMENTAL TABLE 1

Table S1. List of MD simulations of the 5-HT_{2B} receptor. Relates to Figure 4 and Figure S2. Simulations in conditions A–D were initiated from the LSD-bound structure presented in this paper (but, in some cases, with LSD removed or with residue L209^{EL2} mutated to alanine). Simulations in condition E were initiated from a previously published ergotamine-bound crystal structure (PDB: 4NC3).

Condition	Ligand	Mutation	Length (μs)
A	LSD	none	1.1, 5.5, 5.9, 3.4, 2.6, 3.0, 1.2, 1.1, 1.1
B	none	none	1.1, 6.7, 3.9, 2.7, 2.6, 3.3, 1.4, 1.5, 1.4
C	LSD	L209A	1.1, 3.4, 5.9, 5.7, 2.1, 2.7, 2.4, 1.1, 1.1
D	none	L209A	3.0, 3.0, 2.9
E	none (starting from ERG-bound crystal structure; PDB: 4NC3)	none	3.4, 4.7, 4.0, 2.9, 3.1, 3.9

SUPPLEMENTAL TABLE 2**Table S2. Docking scores of LSD and its derivatives against 5-HT_{2B}R structure and 5-HT_{2A}R homology model.** Relates to Figure 3 and Figure S4.

Compound	Docking Score	
	5-HT _{2B} R xray	5-HT _{2A} R model
LSD	-	-68.44
SSAz	-63.73	-65.31
RRAz	-65.32	-62.02
LSA	-58.02	-61.88

SUPPLEMENTAL TABLE 3

Table S3. Wild-type 5-HT_{2B}R and L209A^{EL2} mutant Gq calcium flux and β-Arrestin2 recruitment activity. Relates to Figures 4 and 5. Data were acquired by Gq FLIPR calcium flux and Tango arrestin assays conducted in parallel. EC₅₀ and Emax estimates represent the average and standard error of the mean (SEM) from three independent experiments performed in triplicate. Emax is defined as percent 5-HT maximum response.

Ligand	5-HT _{2B} R				5-HT _{2B} R L209A ^{EL2}			
	Gq-Calcium		β-Arrestin2		Gq-Calcium		β-Arrestin2	
	EC ₅₀ , nM (pEC ₅₀ ± SEM)	Emax %	EC ₅₀ , nM (pEC ₅₀ ± SEM)	Emax %	EC ₅₀ , nM (pEC ₅₀ ± SEM)	Emax %	EC ₅₀ , nM (pEC ₅₀ ± SEM)	Emax %
5-HT	1.43 (8.86 ± 0.04)	100	6.5 (8.32 ± 0.13)	100	1.67 (8.85 ± 0.12)	100	63 (7.34 ± 0.12)	100
LSD	34 (7.52 ± 0.09)	73 ± 4	0.68 (9.18 ± 0.07)	62 ± 4	12.8 (8.03 ± 0.11)	57 ± 9	34 (7.63 ± 0.13)	45 ± 5
ERG	191 (6.79 ± 0.13)	84 ± 2	2.6 (8.60 ± 0.06)	109 ± 5	110 (7.20 ± 0.21)	90 ± 2	14.6 (8.00 ± 0.20)	95 ± 6
LSA	115 (6.95 ± 0.06)	55 ± 5	54 (7.40 ± 0.18)	40 ± 3	79 (7.17 ± 0.19)	40 ± 14	1160 (6.14 ± 0.17)	48 ± 9
SSAz	58.4 (7.26 ± 0.09)	74 ± 3	0.4 (9.38 ± 0.03)	57 ± 5	18.2 (7.86 ± 0.22)	65 ± 7	3.4 (8.53 ± 0.14)	44 ± 3
RRAz	85 (7.10 ± 0.08)	53 ± 6	3.3 (8.54 ± 0.12)	36 ± 5	40.5 (7.45 ± 0.15)	37 ± 13	69 (7.51 ± 0.35)	38 ± 5
(+)-Norfen	10.3 (8.08 ± 0.17)	97 ± 1	20 (7.70 ± 0.06)	78 ± 1	170 (6.99 ± 0.13)	95 ± 6	1270 (6.22 ± 0.30)	79 ± 3
Ro 60-0175	3.36 (8.53 ± 0.16)	97 ± 2	0.45 (9.44 ± 0.10)	75 ± 8	5.3 (8.44 ± 0.21)	94 ± 5	1.75 (8.76 ± 0.01)	67 ± 2
BW723C86	4.51 (8.38 ± 0.09)	97 ± 2	11.9 (8.05 ± 0.11)	82 ± 4	0.93 (9.24 ± 0.15)	96 ± 5	1.10 (9.19 ± 0.03)	85 ± 1

SUPPLEMENTAL TABLE 4

Table S4. Wild-type 5-HT_{2A}R and L229A^{EL2} mutant Gq calcium flux and β-Arrestin2 recruitment activity. Relates to Figures 4 and 5. Data were acquired by Gq FLIPR calcium flux and Tango arrestin assays conducted in parallel. EC₅₀ and Emax estimates represent the average and standard error of the mean (SEM) from three independent experiments performed in triplicate. Emax is defined as percent 5-HT maximum response.

Ligand	5-HT _{2A} R				5-HT _{2A} R L229A ^{EL2}			
	Gq-Calculum		β-Arrestin2		Gq-Calculum		β-Arrestin2	
	EC ₅₀ , nM (pEC ₅₀ ± SEM)	Emax %	EC ₅₀ , nM (pEC ₅₀ ± SEM)	Emax %	EC ₅₀ , nM (pEC ₅₀ ± SEM)	Emax %	EC ₅₀ , nM (pEC ₅₀ ± SEM)	Emax %
5-HT	0.65 (9.25 ± 0.11)	100	133 (6.89 ± 0.07)	100	17.5 (7.85 ± 0.11)	100	1910 (8.84 ± 0.03)	100
LSD	3.61 (8.45 ± 0.09)	86 ± 4	0.52 (9.35 ± 0.11)	60 ± 4	4.70 (8.37 ± 0.07)	75 ± 5	10.2 (8.00 ± 0.10)	42 ± 9
ERG	5.4 (8.32 ± 0.15)	84 ± 3	3.06 (8.60 ± 0.19)	75 ± 4	12.6 (7.99 ± 0.12)	83 ± 3	15.3 (7.84 ± 0.11)	93 ± 7
LSA	17.4 (7.79 ± 0.12)	82 ± 3	58 (7.29 ± 0.12)	47 ± 4	72 (7.18 ± 0.11)	62 ± 6	482 (6.37 ± 0.16)	50 ± 8
SSAz	3.48 (8.49 ± 0.09)	84 ± 4	0.92 (9.06 ± 0.10)	56 ± 3	5.09 (8.34 ± 0.16)	75 ± 4	10.1 (8.00 ± 0.02)	60 ± 2
RRAz	4.17 (8.42 ± 0.12)	84 ± 4	3.70 (8.45 ± 0.09)	57 ± 4	7.85 (8.15 ± 0.15)	77 ± 5	66 (7.18 ± 0.06)	52 ± 6

SUPPLEMENTAL TABLE 5

Table S5. Time-dependent LSD log(τ/K_A) estimates of IP accumulation and β -Arrestin2 BRET translocation. Relates to Figure 5. Data were acquired by PI hydrolysis assays measuring IP accumulation and by BRET assays measuring β -Arrestin2 recruitment. Estimates of log(τ/K_A) represent the average and standard error of the mean (SEM) from three independent experiments performed in duplicate.

Time (min)	5-HT _{2B} R		5-HT _{2B} R L209A ^{EL2}		5-HT _{2A} R		5-HT _{2A} R L229A ^{EL2}	
	IP	β -Arr2	IP	β -Arr2	IP	β -Arr2	IP	β -Arr2
5	8.25 ± 0.28	7.53 ± 0.20	7.84 ± 0.31	8.02 ± 0.13	8.28 ± 0.10	7.88 ± 0.04	8.06 ± 0.17	8.70 ± 0.07
30	8.85 ± 0.09	8.44 ± 0.02	8.60 ± 0.18	8.19 ± 0.01	9.54 ± 0.10	8.44 ± 0.24	9.52 ± 0.01	8.82 ± 0.04
60	9.43 ± 0.06	8.89 ± 0.03	9.04 ± 0.01	8.27 ± 0.01	9.96 ± 0.10	9.28 ± 0.11	9.85 ± 0.07	8.65 ± 0.01
120	9.77 ± 0.01	9.05 ± 0.05	9.33 ± 0.23	8.14 ± 0.02	10.44 ± 0.06	9.54 ± 0.01	10.19 ± 0.11	8.19 ± 0.05
300	10.52 ± 0.03	9.19 ± 0.11	9.70 ± 0.02	8.27 ± 0.05	11.01 ± 0.01	9.50 ± 0.03	10.96 ± 0.23	8.30 ± 0.03